Risk Assessment of Thyroid Follicular Cell Tumors

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Thyroid follicular cell tumors arise in rodents from mutations, perturbations of thyroid and pituitary hormone status with increased stimulation of thyroid cell growth by thyroid-stimulating hormone (TSH), or a combination of the two. The only known human thyroid carcinogen is ionizing radiation. It is not known for certain whether chemicals that affect thyroid cell growth lead to human thyroid cancer. The U.S. Environmental Protection Agency applies the following science policy positions: 1) chemically induced rodent thyroid tumors are presumed to be relevant to humans; 2) when interspecies information is lacking, the default is to assume comparable carcinogenic sensitivity in rodents and humans; 3) adverse rodent noncancer thyroid effects due to chemically induced thyroid–pituitary disruption are presumed to be relevant to humans; 4) linear dose–response considerations are applied to thyroid cancer induced by chemical substances that either do not disrupt thyroid functioning or lack mode of action information; 5) nonlinear thyroid cancer dose–response considerations are applied to chemicals that reduce thyroid hormone levels, increase TSH and thyroid cell division, and are judged to lack mutagenic activity; and 6) nonlinear considerations may be applied in thyroid cancer dose–response assessments on a case-by-case basis for chemicals that disrupt thyroid–pituitary functioning and demonstrate some mutagenic activity. Required data for risk assessment purposes is mode of action information on mutagenicity, increases in follicular cell growth (cell size and number) and thyroid gland weight, thyroid–pituitary hormones, site of action, correlations between doses producing thyroid effects and cancer, and reversibility of effects when dosing ceases. *Key words*: iodide pump, microsomal enzyme induction, 5′-monodeiodinase, risk assessment, thyroid follicular cell tumors, thyroid hormone (T_4, T_3), thyroid peroxidase, thyroid-stimulating hormone (TSH), UDP glucuronosyl transferase. *Environ Health Perspect* 106:447–457 (1998). [Online 29 June 1998]
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The U.S. Environmental Protection Agency (EPA) conducts risk assessments on chemicals for carcinogenicity under the guidance provided in its cancer assessment guidelines (*1,2*). From time to time, scientific developments cause the agency to reexamine procedures that are generally applied. That is the case with the review of some chemicals that have produced thyroid follicular cell tumors in experimental animals. In this paper, we will present a summary of thyroid carcinogenesis and describe the procedures the EPA will use to evaluate these tumors and the data that are needed to make these judgments. Experience in applying the thyroid policy to a set of pesticides has been reported (*3*).

The proposed EPA cancer assessment guidelines (*2*) generally operate on the rebuttable premise that findings of chemically induced cancer in laboratory animals signal potential hazards in humans. Likewise, for dose–response analyses, the guidelines first call for use of the most biologically appropriate means for dose extrapolation. In the absence of such knowledge, assessors are directed toward the use of default science policy positions, either low-dose linear, nonlinear, or both procedures. Male rat kidney tumors due to accumulation of α2u-globulin were the first instance in which the EPA developed generic guidance for the assessment of a specific tumor type (*4*); thyroid tumors are the second.

Although the present effort is directed specifically at tumors of the thyroid follicular cells, it is recognized that endocrine organs that secrete hormones into the blood or lymph respond to stimuli via common pathways, and other organs may develop tumors by disruption of hormonal balance. Therefore, it may be possible that some of the lessons learned with the analysis of thyroid follicular cell tumors will be applicable to other endocrine tumor sites in the future (*5,6*).

In 1988, the EPA developed a review of the existing science on thyroid follicular cell carcinogenesis and a draft science policy position covering the evaluation of chemicals that have induced thyroid tumors in experimental animals. The external EPA Science Advisory Board approved the science review (*7*) and tentatively embraced the policy position that dose–response relationships for some thyroid tumors could be assessed using nonlinear considerations. After revision and a second review, the policy document was finalized (*8*). This paper presents the underlying science policy for thyroid follicular cell tumors.

Overview of Thyroid Carcinogenesis

Circulating thyroid hormone determines the level of operation of most cells of the body (*9*); too much or too little hormone results in disease. Control of the concentration of this endocrine hormone in the blood is mainly regulated by a negative feedback involving three organs: the thyroid gland, which produces thyroid hormone, and the pituitary gland and hypothalamus, which respond to and help maintain optimal levels of thyroid hormone (Fig. 1). The hypothalamus stimulates the pituitary through thyrotropin-releasing hormone (TRH) to produce thyroid-stimulating hormone (TSH), which then prompts the thyroid gland to produce thyroid hormone. The stimulated thyroid actively transports inorganic iodide into the follicular cell and converts it to an organic form and then into thyroid hormone molecules, which can influence target organs throughout the body. Thyroid hormone, in tissues peripheral to the thyroid, can be converted from a less active thyroxine (T_4) to a more active triiodothyronine (T_3) form. Thyroid hormone is also metabolized by the liver, largely by conjugation reactions, and excreted into the bile.

Cells in the hypothalamus and pituitary gland respond to levels of circulating thyroid hormone, such that when thyroid hormone levels are high, there is a signal to reduce the output of TRH and TSH. Likewise, when thyroid hormone levels are reduced, the

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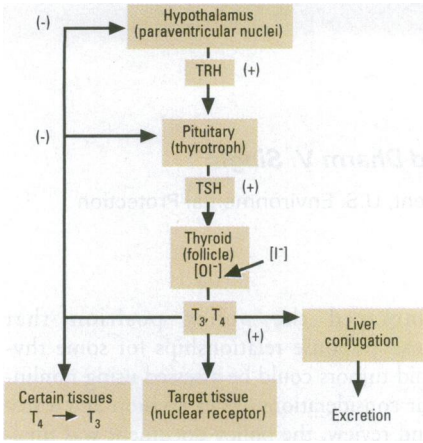

Figure 1. Hypothalamic–pituitary–thyroid axis. Abbreviations: TRH, thyrotropin-releasing hormone; TSH, thyroid stimulating hormone; T_3, triiodothyronine; T_4, thyroxine.

hypothalamus and pituitary are prompted to deliver more TSH to the thyroid gland to increase the output of thyroid hormone. This negative feedback loop helps the body to respond to varying demands for thyroid hormone and to maintain hormone homeostasis. Circulating T_4, T_3, and TSH can readily be monitored in the serum of experimental animals and humans by radioimmunoassay and serve as biomarkers of exposure and effect of agents that disrupt thyroid–pituitary status.

In mammals, when demands for more thyroid hormone are small, existing thyroid follicular cells can meet the demand. With increased need, as a result of certain chemical exposures or iodide deficiency, the thyroid responds by increasing the size (hypertrophy) and number (hyperplasia) of thyroid follicular cells to enhance hormone output. With continued TSH stimulation there is actual enlargement of the thyroid gland (goiter) and, at least in rodents, eventually neoplasia of the thyroid follicular cells. Since TSH-producing pituitary cells are also stimulated, they too sometimes undergo hyperplasia and neoplasia.

For details about thyroid follicular cell carcinogenesis, consult Hill et al. (*7*) and an update of the science since 1988 (*10*), as well as a number of relevant reviews (*11–25*). Rich historical literature is also valuable (*26–33*).

Role of TSH in Rodent Carcinogenesis

Several experimental findings in rodents that perturb thyroid–pituitary homeostasis and lead to elevated TSH levels indicate the central role of TSH in inducing thyroid carcinogenic effects: 1) loss of thyroid cells through partial thyroidectomy led to a sustained inability of the thyroid to meet the demands for thyroid hormone; 2) iodide deficiency decreased the thyroid's ability to synthesize adequate supplies of thyroid hormones; and 3) transplantation of pituitary tumors that autonomously secrete TSH added more of the trophic hormone to graft recipients (*7*). These experimental manipulations were done in the absence of any exogenous chemical treatment but demonstrate the seminal qualitative role that TSH plays in thyroid carcinogenesis. Quantitatively, its significance is demonstrated by the correlation between the TSH level and the number of tumors/gland in an initiation–promotion study (*15*).

If a goitrogenic stimulus that would lead to thyroid tumor formation in rodents is removed early in the process, effects reverse toward normal (*34*). Likewise, if a goitrogenic stimulus is given in conjunction with adequate amounts of exogenous thyroid hormone (*35*) or after hypophysectomy to remove TSH-secreting cells (*36*), then hypertrophy, hyperplasia, and tumors of the thyroid do not develop. It follows from these observations that if TSH levels are chronically elevated, there will be thyroid cell hypertrophy, hyperplasia, and some potential for neoplasia, but under conditions where thyroid–pituitary homeostasis is maintained, the steps leading to tumor formation are not expected to develop, and the chances of tumor development are negligible.

Genetic Influences

Rodent studies indicate an interplay between genetic and nongenetic events in the development of thyroid tumors. Evidence indicates that carcinogenesis often proceeds through a number of operational steps: initiation, promotion, and progression (*37*). Initiation seems linked to genetic events with the induction of DNA mutations, whereas promotion includes at least nongenetic events that lead to the expansion of a clone of initiated cells via repeated cell division. Progression is associated with the accumulation of cell behaviors (such as enzymatic destruction of basement membranes and increased mobility) that allow cells to invade locally and metastasize distally, probably in part due to still other mutations. Some genetic influences do not result in mutations, but in changes in gene expression that can affect the carcinogenic process.

Treatment regimens that produce thyroid tumors in rodents can be conceptualized in regard to initiation and promotion. These steps do not themselves describe carcinogenic mechanisms but, instead, present a framework for viewing experimental findings.

- In two-stage experiments in which a mutagenic agent such as radioactive iodide is followed by treatment with a nonmutagenic goitrogen (e.g., a chemical inhibitor of thyroid hormone synthesis), the first agent acts like an initiator and the second behaves as a promoter (*38*).
- When treatment with a goitrogen alone leads to tumor formation, TSH increases cell division among normal cells, which leads to increases in the overall chance of a spontaneous initiating mutation, and then promotes the altered cells, which retain responsiveness to TSH; carcinogenesis in these cases would be free of chemically induced mutagenic effects (*39*).
- Some chemicals appear to have both initiating and promoting activity, as they are mutagenic in many test systems and have significant antithyroid activity (e.g., 4,4′-oxydianiline) (*40*).
- Still other agents, such as x irradiation (*41*) and certain chemicals (e.g., some nitrosamines) (*42*), are definitely mutagenic but lack intrinsic goitrogenic activity.

These agents can easily initiate the carcinogenic process, but tumor formation would be independent of strong promotional activity from antithyroid effects. TSH may still play a permissive role, as these agents can induce cell injury and cell death, which lead to reductions in the output of thyroid hormone and increases in TSH-induced cell division of initiated thyroid cells.

It thus appears that thyroid cancer in experimental animals may be due to mutagenic influences that lead to DNA changes (X rays, ^{131}I, mutagenic chemicals), to hormone perturbation that leads to growth stimulation, or both. Hormone perturbation directly increases the number of thyroid cells and indirectly leads to mutations (partial thyroidectomy, transplantation of TSH-secreting pituitary tumors, iodide deficiency, chemicals inhibiting iodide uptake by the thyroid, chemicals inhibiting thyroid peroxidase, chemicals inhibiting release of thyroid hormone from the thyroid gland, chemicals damaging thyroid follicular cells, chemicals inhibiting conversion of T_4 to T_3, chemicals increasing hepatic thyroid hormone metabolism and excretion). In those cases where increases in cell number are dominant, the inciting agent or procedure may be seen as the carcinogenic stimulus, but the proximate carcinogenic influence is TSH. For those with a dominant mutagenic influence, TSH may play an enhancing role in the carcinogenic process.

Possible Mechanistic Steps

The precise molecular steps in the carcinogenic process leading to thyroid follicular cell cancer have not been totally elucidated, although significant insights into the problem

have been described (*25,43*). Normal cell division in the thyroid seems to be affected by an interplay among several mitogenic factors, namely TSH, insulin-like growth factor 1 (IGF-1), insulin, epidermal growth factor (EGF), and possibly fibroblast growth factor (FGF). Still other factors such as transforming growth factor β (TGFβ), certain interferons, and interleukin 1 may inhibit growth.

TSH communicates with the cell's interior by activating adenylate cyclase to raise levels of cyclic AMP. It also functions through the phosphatidyl-inositol/Ca^{2+} signal transduction cascade, which activates phospholipase C. This latter system expresses itself through two pathways: inositol triphosphate, which releases calcium from cellular stores, and 1,2-diacylglycerol, which activates protein kinase C. Signal transduction continues following protein kinase C activation through several steps, including the *ras* protooncogene and various kinases, culminating in the activation of nuclear transcription factor genes (e.g., c-*fos*), which leads to cellular proliferation. The diacylglycerol pathway may account for the fact that the phorbol ester tumor promoters, which increase protein kinase C, also stimulate thyroid cell division.

EGF, insulin, and IGF-1 act through tyrosine kinase receptors. TSH increases EGF binding to its receptor and enhances cell division. IGF-1 and high doses of insulin may influence the TSH receptor. Iodide decreases thyroid cell adenylate cyclase and calcium levels, and reduced iodide enhances TSH effectiveness. In sum, the actual control of normal cell division in follicular cells may, in fact, represent some interaction of all these factors and possibly other growth regulatory substances.

Under conditions of thyroid–pituitary imbalance, there is no question that TSH plays a significant role in stimulating DNA synthesis and cell proliferation; however, there is a controversy concerning the extent that normal follicular cells can proliferate. One research group claims that cells have limited capacity to respond to the growth-inducing effects of TSH (*44*). In this case, tumor formation would entail mutational steps that free cells from their growth limiting potential. Another group of investigators thinks that follicular cells are innately heterogeneous, with some of them having stem cell-like proliferation potential while others are more restricted (*45*). The stem cell-like follicular cells would continue to respond to TSH stimulation and eventually give rise to tumors. It seems possible that both positions might actually apply.

Neoplastic transformation appears to occur in single cells that then expand clonally. Under TSH stimulation, the yield of mutations that may influence transformation increases, even in the absence of an increase in the mutation rate per cell. This is because the repeated cell divisions lead to an increased number of cells at risk for mutation, or rapid cell turnover leaves some spontaneous DNA damage unrepaired.

The precise genetic alterations that accumulate in thyroid follicular cells have not been clearly established in humans or in experimental systems, although mutations involving the *ras* protooncogene, the *p53* tumor suppressor gene, and various chromosome aberrations have been reported in the follicular variety of epithelial tumors. These changes in gene expression could lead to uncontrolled cellular growth and allow cells to attain the ability to invade adjacent tissues and metastasize (Fig. 2). For the papillary variety of thyroid epithelial tumors, changes in expression of other factors have been noted, namely protooncogenes PTC/*ret*, *trk*, and *met* (*25,43*).

Transformed rodent cells that are stimulated to proliferate under the influence of continuing antithyroid stimulation retain their responsiveness to TSH. Interestingly, human thyroid cancer cells often retain TSH receptors and the ability to respond to TSH, although their receptors dissipate as tumors become more anaplastic. Tumor cells that attain maximal malignant potential usually lose their dependence on TSH. Although interesting observations concerning growth regulation associated with thyroid carcinogenesis have been made, clearly more work is needed.

Human Thyroid Carcinogenesis

Clinically manifest thyroid cancer in humans in the United States is uncommon and largely nonfatal; only about 17,200 new cases occur each year (an incidence rate of around 3 per 100,000 persons, with about 1,200 deaths annually (>90% 5-year survival, which constitutes only around 0.5% of all cancer deaths) (*46,47*). In contrast to clinically apparent disease, small occult thyroid cancers are noted at autopsy in a small percentage of persons in a number of surveys and up to about 50% in other investigations (*48,49*). The incidence in autopsy studies is more like that noted in rats in the National Toxicology Program, where about 0.4% of control rats are diagnosed with thyroid cancers at 2 years of age (*50*). However, this comparison is somewhat misleading. Detailed histological examinations of human and rodent thyroids are not routinely performed by all investigators. Histological criteria for tumors differ over time and across reviewers. In addition, thyroid follicular cell cancer in humans is most often diagnosed histologically as a papillary pattern and less often as a follicular pattern,

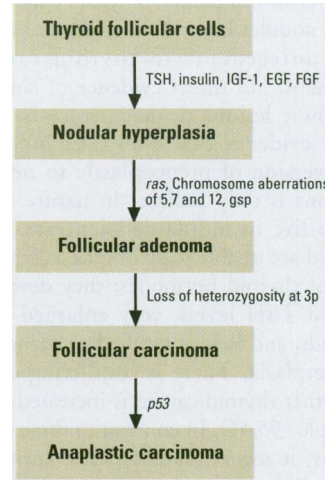

Figure 2. Possible molecular events in human thyroid (follicular) carcinogenesis. Abbreviations: TSH, thyroid-stimulating hormone; IGF-1, insulin-like growth factor 1; EGF, epidermal growth factor; FGF, fibroblast growth factor; gsp, GTP-binding protein mutation.

whereas in rodents the follicular cell tumors are of a follicular pattern. The aggressiveness of tumors varies: rodent thyroid neoplasms rarely metastasize; human cancers frequently metastasize. These differences regarding histology, along with the shortcomings of information from descriptive and analytical epidemiologic investigations, help to emphasize the difficulty in comparing human and rodent cancer incidence data.

For years, the only known human thyroid carcinogen was x irradiation, which caused an increase in papillary tumors (*41,51*). It was questioned whether ionizing radiation from diagnostic or therapeutic use of ^{131}I was carcinogenic in humans (*52,53*), although more recently, children exposed to radioiodine (^{131}I) following the Chernobyl reactor accident in the Ukraine have developed thyroid cancer; iodide deficiency is also common in the region and may augment the response to ^{131}I (*54*). Most of the human chemical carcinogens appear to be mutagenic and cause tumors in more than one site; some are steroid hormones. To date, no chemical has been identified as being carcinogenic to the human thyroid.

Humans respond as do experimental animals in regard to short- and mid-term disturbances in thyroid functioning from various antithyroid stimuli such as iodide deficiency, partial thyroidectomy (surgically or ^{131}I induced), and goitrogenic chemicals (e.g., thionamides): when circulating thyroid hormone levels go down, the TSH level rises and induces thyroid hypertrophy and hyperplasia.

However, the long-term consequences of antithyroid action are harder to interpret, and there is controversy regarding whether the enlarged human thyroid gland undergoes

conversion to cancer. Thyroid enlargements and nodules have been implicated as possible antecedents to thyroid cancer in humans, but direct evidence of conversion of these lesions to malignancy is lacking. The evidence that does exist for possible conversion of preneoplastic to neoplastic lesions is only indirect in nature. Persons who live in iodide-deficient areas of the world are unable to synthesize adequate levels of thyroid hormones; they develop elevated TSH levels, very enlarged thyroid glands, and lesions typified as adenomatous hyperplasia. There is conflicting evidence whether thyroid cancer is increased in these people (*55,56*). In contrast to these observations, it seems that domestic animals but not wild animals in iodide deficient areas developed elevated incidences of thyroid tumors (*57*), and tumor incidence disappeared in dogs following the advent of using iodized salt (*58*). Iodide may have some influence on the histological type of thyroid cancer, with follicular being more common in iodide deficient areas and papillary being more common in iodide rich areas (*59*). People with various inborn errors of metabolism that are unable to synthesize enough effective thyroid hormone develop very enlarged thyroids, but few cases of cancer have been reported. There are no reports of thyroid tumors among persons with resistance to thyroid hormone (*60,61*). In persons with the autoimmune disorder Graves' disease, there are often immunoglobulins that stimulate thyroid cells in ways analogous to TSH, even though TSH levels per se are very low. It is not known whether there is an increase in thyroid cancer among these patients; some studies seem to indicate either that cancer incidence may be increased or that thyroid tumors in these patients may be more aggressive (*62,63*). Overall, this qualitative information suggests that prolonged stimulation of the human thyroid under certain circumstances may lead to cancer, as in the presence of inherited metabolic conditions or long-term immunologic abnormalities, but there is uncertainty in this conclusion.

In epidemiologic studies, goiter and thyroid nodules have been shown to be risk factors for thyroid cancer. The specific causes of these enlargements are not known but, where studied, do not appear to be due to hypothyroidism (*64,65*). Some researchers believe that part of the association may be due to the close medical scrutiny given to persons with suspicious thyroid enlargements (*66,67*).

In spite of the potential qualitative similarities, there is evidence that humans may not be as quantitatively sensitive to thyroid cancer development from thyroid–pituitary disruption as rodents. Rodents readily respond to reduced iodide intake with the development of cancer; humans develop profound hyperplasia with adenomatous changes with only suggestive evidence of malignancy. Even with congenital goiters due to inherited blocks in thyroid hormone production, only a few malignancies have been found in humans.

The reasons for differences in perceived interspecies sensitivity are not fully known. However, one factor that may play a role in interspecies quantitative sensitivity to thyroid stimulation deals with the influence of protein carriers of thyroid hormone in the blood (Table 1). Both humans and rodents have nonspecific low affinity protein carriers of thyroid hormone (e.g., albumin). However, in humans, other primates, and dogs there is a high affinity binding protein, thyroxine-binding globulin, which binds T_4 (and T_3 to a lesser degree); this protein is missing in rodents and lower vertebrates. As a result, T_4 bound to proteins with lower affinity in the rodent is more susceptible to removal from the blood, metabolism, and excretion from the body. In keeping with this finding, the serum half-life of T_4 is much shorter in rats (<1 day) than in humans (5–9 days); this difference in T_4 half-life results in a 10-fold greater requirement for endogenous T_4 in the rat thyroid than in the adult human (*68*). Serum T_3 levels also show a species difference; the half-life is around 6 hr in rats and 24 hr in humans (*69,70*). There is a morphological consequence to these hormone differences. High thyroid hormone synthetic activity is demonstrated in follicles in rodents: they are relatively small, surrounded often by cuboidal epithelium. Follicles in primates demonstrate less activity and are large with abundant colloid, and follicular cells are relatively flattened (low cuboidal) (*16*).

The accelerated production of thyroid hormone in the rat is driven by serum TSH levels that are probably about 6- to 60-fold higher than in humans. This assumes a basal TSH level in rats and humans of 200 ng/ml and 5 μU/ml, respectively, and a potency of human TSH of 1.5–15 U/mg of hormone (*71*). Thus, it appears that the rodent thyroid gland is chronically stimulated by TSH levels to compensate for the increased turnover of thyroid hormone. It follows that increases in TSH levels above basal levels in rats could more readily move the gland toward increased growth and potential neoplastic change than in humans. Interestingly, adult male rats have higher serum TSH levels than females (*72*), and they are often more sensitive to goitrogenic stimulation and thyroid carcinogenesis. In humans, there is no sex difference in hormone levels, but females more frequently develop thyroid cancer (*46*).

In addition to considerations about the influence of serum thyroid hormone carrier proteins, there are differences between humans and animals in size, lifespan, basal metabolic rate, and pharmacokinetics and pharmacodynamics of endogenous and exogenous chemicals. Any comparison of thyroid carcinogenic responses across species should be cognizant of all these factors.

The guidance given here on thyroid tumors is not unique. Other authorities have recognized and incorporated advances in the understanding about carcinogenic mechanisms into their assessments of cancer risks (*73–78*).

Science Policy Guidance

Position Statements

Rodents and humans share a common physiology in regard to the thyroid–pituitary feedback system. Short-term perturbation in this system often leads to similar effects in both groups, resulting in increases and decreases in circulating thyroid and pituitary hormones. It is well established in rodents that disruption of thyroid–pituitary status with elevation of TSH levels is associated with thyroid tumor and sometimes related pituitary tumor development. This is true whether it is due to deficiency in iodide, reduction in thyroid mass, presence of TSH-producing pituitary tumors, or administration of goitrogenic chemicals. An increase in TSH stimulation of the thyroid is a final common development. Likewise, administration of exogenous thyroid hormone or removal of a TSH-increasing stimulus reduces the effects in the thyroid.

The role of thyroid–pituitary disruption in cancer development in humans is much less convincing than in animals. Iodide deficiency has been associated with increases in thyroid cancer in some studies but not in

Table 1. Interspecies and intraspecies differences

Parameter	Human	Rat
Thyroxine binding globulin	Present	Essentially absent
T_4 half-life	5–9 Days	0.5–1 Day
T_3 half-life	1 Day	0.25 Day
T_4 production rate/kg body weight	1×	10× that in humans
Serum TSH	1×	6–60× that in humans
Follicular cell morphology	Low cuboidal	Cuboidal
Sex differences		
Serum TSH	M = F	M ≤ 2 × F
Cancer sensitivity	F = 2.5 × M	M > F

Abbreviations: T_4, thyroxine; T_3, triiodothyronine; TSH, thyroid stimulating hormone; M, male; F, female.

others. Similarly an association between either inborn errors of metabolism affecting thyroid hormone output or autoimmune-related Graves' disease and cancer has been suggested but not proven. TSH may at least play some permissive role in carcinogenesis in humans. Accordingly, we cannot qualitatively reject the animal model; it seems reasonable that it may serve as an indicator of a potential human thyroid cancer hazard. However, to the extent that humans are susceptible to the tumor-inducing effects of thyroid–pituitary disruption and given that definitive human data are not available, humans appear to be quantitatively less sensitive than rodents to developing cancer from perturbations in thyroid–pituitary status. Recognizing these things and based upon thyroid carcinogenesis mode of action considerations, the EPA adopted the following three science policy positions:

1. It is presumed that chemicals that produce rodent thyroid tumors may pose a carcinogenic hazard for the human thyroid.
2. In the absence of chemical-specific data, humans and rodents are presumed to be equally sensitive to thyroid cancer due to thyroid–pituitary disruption. This is a conservative position when thyroid–pituitary disruption is the sole mode of action in rats because rodents appear to be more sensitive to this carcinogenic mode of action than humans. When the thyroid carcinogen is a mutagenic chemical, the possibility that children may be more sensitive than adults needs to be evaluated on a case-by-case basis.
3. Adverse rodent noncancer thyroid effects (e.g., thyroid gland enlargements) following short- and long-term reductions in thyroid hormone levels are presumed to pose human noncancer health hazards.

Some chemicals that have produced thyroid follicular cell tumors in laboratory rodents appear to work by producing a disturbance in thyroid–pituitary homeostasis, others appear to act primarily through a mutagenic mode of action, and still others seem to show a combination of both modes of action. The question then becomes how to evaluate the risks of thyroid tumors for humans given exposure to any of these chemicals. If the animal tumors are due to chemical doses that produce imbalances in thyroid–pituitary functioning, the chance of cancer is anticipated to be minimal under conditions of hormonal homeostasis. Tumors that seem to arise from relevant mutagenic influences (e.g., gene mutations and structural chromosome aberrations) without perturbation in thyroid–pituitary status may pose some chance of cancer across a broader range of doses. Consequently, until such time that biologically based models and data become available, the EPA has adopted five other science policy positions for conducting dose–response assessments of chemical substances that have produced thyroid follicular cell (and related pituitary) tumors in experimental animals:

1. A linear dose–response procedure should be assumed when needed experimental data to understand the cause of thyroid tumors are absent and the mode of action is unknown (Table 2, Example 1).
2. A linear dose–response procedure should be assumed when the mode of action underlying thyroid tumors is judged to involve mutagenicity alone (Table 2, Example 2).
3. A nonlinear dose–response relationship (margin of exposure) should be used when thyroid–pituitary disruption is judged to be the sole mode of action of the observed thyroid and related pituitary tumors (Table 2, Example 3). Thyroid–pituitary perturbation is not likely to have carcinogenic potential in short-term or highly infrequent exposure conditions. The margin of exposure procedure generally should be based on thyroid–pituitary disruptive effects themselves, in lieu of tumor effects, when data permit. Such analyses will aid in the development of combined noncancer and cancer assessments of toxicity. Results of the margin of exposure procedure will be presented in a way that supports risk management decisions for exposure scenarios of differing types (e.g., infrequent exposure, short durations).
4. Consistent with EPA risk characterization principles, both linear and margin of exposure considerations should be assumed when both mutagenic and thyroid–pituitary disruption modes of action are judged to be potentially at work (Table 2, Example 4). The weight of evidence for emphasizing one over the other should also be presented. The applicability of each to different exposure scenarios should be developed for risk management consideration.
5. Dose–response relationships for neoplasms other than the thyroid (or pituitary) should be evaluated using mode of action information bearing on their induction and principles laid out in current EPA cancer risk assessment guidelines. There is an association between thyroid and liver tumors in rodent cancer studies (*79,80*). The reason(s) for this relationship has not been generically established but should be carefully assessed for chemicals on a case-by-case basis. Some may be due to induction of hepatic microsomal enzymes.

Table 2. Default dose–response procedures for thyroid carcinogens

Array of data			Dose–response methodology
Example	Antithyroid	Mutagenic	
1	Unknown	Unknown	Linear
2	No	Yes	Linear
3	Yes	No	Nonlinear (MOE)
4	Yes	Yes	Linear and nonlinear (MOE)

MOE, margin of exposure.

Most of the focus in implementing this policy is devoted to answering the following questions: 1) Does an agent that shows thyroid carcinogenic effects have antithyroid activity? 2) Can modes of action other than thyroid–pituitary disruption account for thyroid tumor formation by this chemical? and 3) How can one express thyroid dose–response relationships? Adequately answering these questions is dependent upon a data-rich information base for the chemical under review. To the extent practicable, effort should be made to review such information before deciding upon the possible mode of chemical action underlying the thyroid tumors and their consequences for risk assessment.

The procedures and considerations developed in this report embody current scientific knowledge of thyroid carcinogenesis and evolving science policy positions. Should significant new information become available that would change these positions, the EPA will update its science policy positions accordingly.

Evidence for Antithyroid Activity

Data needs. Different types of information on a chemical may indicate that it has antithyroid activity, that is, whether it works via disruption of thyroid–pituitary status. These include effects manifest in the thyroid gland per se, various tissues peripheral to the thyroid, and/or the liver. All available factors are assembled into an overall evaluation of the likelihood that the chemical is acting via disruption of the thyroid–pituitary axis. A number of chemicals have been assessed (*3*).

Special mechanistic studies are needed to demonstrate chemically induced perturbations in thyroid–pituitary functioning. Repeat dose (e.g., 2–4 week and 13 week) studies that have simultaneously evaluated a number of end points can often provide critical information for evaluating qualitatively whether antithyroid activity exists, the cause of the activity, and quantitatively

what dose–response relationships may be appropriate. Such studies should be carefully designed to encompass multiple doses from above those clearly associated with tumors in chronic studies down to those below which there is no indication of disturbance in critical thyroid–pituitary parameters, so that dose–response relationships can be defined. Special attention should be given to the time of sampling of thyroid and pituitary hormones because of the compensatory action of homeostatic mechanisms and the difficulty in discerning changes after compensation occurs. Hormone sampling should also be conducted at the same time during the course of a day to minimize circadian hormonal fluctuations, and efforts should minimize stress in handling animals. Effects measured only at the end of chronic rodent studies are often difficult to evaluate and, alone, seldom provide adequate information.

The determination of the antithyroid activity of a chemical requires empirical demonstration of the following items. Demonstration of increases in thyroid growth and changes in relevant thyroid and pituitary hormones are considered to be the most important. Location of the site(s) of antithyroid action documents where in the body the chemical under assessment leads to perturbations in thyroid–pituitary functioning. Dose correlations among various effects determine where the growth curve for the thyroid gland deviates from the normal pattern of cell replacement and how this relates to doses producing tumors. Reversibility of effects following treatment cessation during the early stages of disruption of the thyroid–pituitary axis shows that permanent, self-perpetuating processes have not been set into motion.

The following three items are desirable in determining antithyroid activity: lesion progression, structure–activity analysis, and other studies. Each provides supporting information that can add profoundly to the assessment of an agent's ability to produce antithyroid effects.

Increases in cellular growth (evidence required). Agents that affect thyroid–pituitary function may stimulate thyroid enlargement. Most parameters commonly measured include, but are not limited to, increases in absolute or relative thyroid gland weight or histological indications of cellular hypertrophy and hyperplasia, morphometric documentation of alteration in thyroid cellular components, and changes in the proliferation of follicular cells detected by DNA labeling or mitotic indices.

Hormone changes (evidence required). With a disruption in thyroid–pituitary functioning, there is typically a reduction in both circulating serum T_4 and T_3 concentrations and an increase in TSH levels within days or a few weeks of chemical administration. In some cases, T_4 levels may be lowered while T_3 levels are maintained within normal limits. In addition, sometimes hormone levels may return to normal over time for mild goitrogenic agents because of the homeostatic compensatory increase in thyroid activity and mass. Statistical tests can help evaluate the significance of hormone perturbations, but it is the constellation of changes in both thyroid and pituitary hormones that indicate whether the negative feedback loop between the thyroid and hypothalamus/pituitary has been perturbed.

Site of action (evidence required). Chemicals that produce thyroid tumors alone or after administration of a mutagenic initiator produce interference with thyroid–pituitary function at various sites in the body (Fig. 3). Effects have been found at one or more of the following anatomical locations: intrathyroidal and various extrathyroidal sites, including the liver and other sites. Clues to the site of action can sometimes be deduced by analysis of structurally related compounds. Generally, enough information on a chemical should be given to be able to identify the sites that contribute the major effect on thyroid–hypothalamus–pituitary function. Given experience to date, it appears that most often the liver is the site of action, followed by the thyroid, where thyroid peroxidase is affected; other sites of action seem to be less common.

Intrathyroidal. Several different effects in the thyroid gland have been associated with the development of antithyroid activity and the formation of thyroid tumors in rodents. Iodide pump inhibition by chemicals like thiocyanate and perchlorate ions leads to a decrease in uptake of inorganic iodide into the thyroid gland. Thyroid peroxidase inhibition blocks the incorporation of active iodide into iodotyrosines and their coupling to form the nascent thyroid hormones. Agents that are known to reversibly or irreversibly inactivate this enzyme include various thionamides such as 6-propylthiouracil and ethylene thiourea, certain aromatic amines, e.g., some of the sulfonamides, and miscellaneous compounds such as amitrole. Toxicity to thyroid cells, as has been seen with polychlorinated biphenyls, may affect the gland's ability to manufacture and secrete thyroid hormones. Inhibition of thyroid hormone release, with agents like lithium and excess iodide, results in a retention of hormones within the colloid and a paucity released into the circulation (*81*).

Extrathyroidal. Several tissues and organs of the body, including skeletal muscle, kidneys, and liver, contain different deiodinases that remove iodine atoms from thyroid hormones. Inhibition of 5′-monodeiodinase, the enzyme that normally converts T_4 to T_3, leads to a reduction in circulating T_3 and an increase in the rT_3 level via 3′-deiodination. Compounds like FD&C Red No. 3 (erythrosine), iopanoic acid, and 6-propylthiouracil act by competitive inhibition of this enzyme or interaction with its sulfhydryl cofactor. The deiodinase system in the pituitary is somewhat different from that in the periphery and may respond differently to certain chemicals (*82*).

A significant amount of thyroid hormone normally is metabolized by the liver. Certain chemicals induce liver microsomal enzymes and enhance thyroid hormone metabolism and removal. T_4 conjugation with glucuronic acid is enhanced by agents that induce hepatic glucuronosyl transferase (*19*). In these cases, thyroid hormone may also show increased binding to hepatocytes, increased biliary excretion, and increased plasma clearance. Other common manifestations of microsomal induction include enlargement of hepatocytes in the centrolobular region, an increase in hepatic cell smooth endoplasmic reticulum, an increase

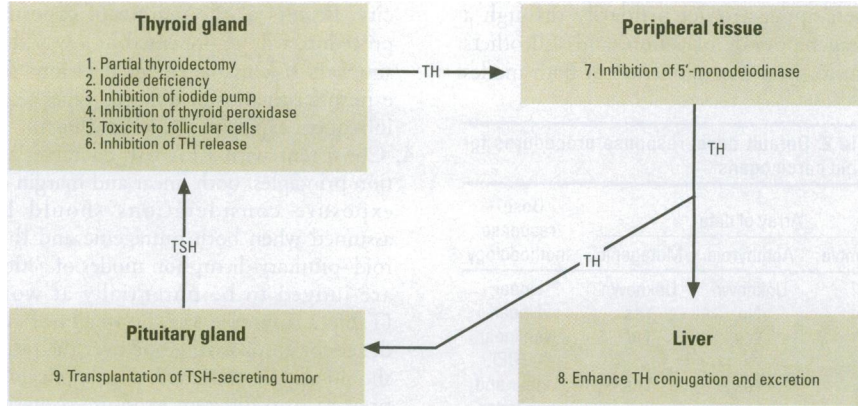

Figure 3. Antithyroid effects that influence thyroid carcinogenesis. Abbreviations: TH, thyroid hormone; TSH, thyroid stimulating hormone.

in P450-associated metabolism of various chemical substrates, and an increase in biliary flow.

Disparate chemical and functional classes like polyhalogenated hydrocarbons (e.g., 2,3,7,8-tetrachlorodibenzo-*p*-dioxin), barbiturates (e.g., phenobarbital), and various individual compounds (e.g., the pesticide clofentezine, the drug spironolactone, and the histamine antagonist SK&F 934790) are known to enhance thyroid hormone excretion via effects on microsomal enzymes. Conjugation also may occur with sulfate, usually associated with deiodination; deamination, oxidative decarboxylation, and ether-link cleavage are minor degradative pathways. Interestingly, phenobarbital has been shown to be a promoter in the rodent thyroid (*35*), but there is no indication it produces thyroid cancer in humans (*83*).

Chemicals may bind thyroid hormone receptors and produce certain effects. For instance, agents (e.g., salicylates) can displace thyroid hormone from plasma carrier proteins and result in reductions in effective thyroid hormone (*84,85*). They could bind receptors in target organs (e.g., pituitary) but result in inactive complexes. These possibilities plus other potential sites of action (e.g., affecting TRH, thyroid hormone responsive elements on the DNA) may be conceived as influencing thyroid–pituitary functioning.

Dose correlations (evidence required). Confidence in an antithyroid mode of action is enhanced by evidence of a correlation between doses of a chemical that do and do not jointly perturb thyroid–pituitary hormone levels, produce various histological changes in the thyroid, or produce other effects, including thyroid cancer. These are important steps in evaluating the significance of thyroid–pituitary disruption in thyroid carcinogenesis and in evaluating dose–response relationships.

Reversibility (evidence required). Chemicals working through an antithyroid mode of action induce changes in thyroid cell morphology and number and in thyroid–pituitary hormones that are reversible upon cessation of chemical dosing.

Lesion progression (evidence desirable). Evidence for a progression of histological lesions over time is commonly noted during the carcinogenic process. These include cellular hypertrophy and hyperplasia, followed by focal hyperplasia and, eventually, neoplasia (benign and possibly malignant tumors).

Structure–activity analyses (evidence desirable). Analysis of chemical structure may show that an agent belongs to a class of compounds that induces thyroid tumors via thyroid–pituitary imbalance (e.g., agents that inhibit thyroid peroxidase, liver microsomal enzyme inducers). This allows for the scientific inference that the chemical under review may act similarly. In addition, generic information developed on a group of analogues can be used to support the assessment of the agent under review.

Other studies (evidence desirable). Many other studies bearing on thyroid–pituitary imbalance can provide a range of findings from strong ancillary information to only suggestive indications. For example, a few of these studies include suppression of induced effects by concurrent administration of thyroid hormone; absence of initiating activity but presence of promoting activity in two-stage carcinogenicity tests; localization of certain chemicals in the thyroid (e.g., thionamides); influences on hypothalamic responsiveness to thyroid hormone levels and output of TRH; changes in TSH mRNA transcripts in the pituitary; and alteration of thyroid hormone nuclear receptor number or synthesis.

Other Modes of Carcinogenic Action

Another critical element in the evaluation of a thyroid carcinogen is a determination of whether mutagenicity may account for the observed tumors. Primary emphasis should be placed on those end points that have mechanistic relevance to carcinogenicity. DNA reactivity is a prime predictor of potential mutagenic carcinogenicity. Many of these compounds belong to particular chemical classes (e.g., aromatic amines, nitrosamines, polyaromatic hydrocarbons). These chemicals or their metabolites bind to DNA, and they often induce gene mutations and structural chromosome aberrations. In recognition that organs and tissues may have unique metabolic activity, it is helpful to know in addition to traditional short-term test results whether there is evidence of DNA reactivity in target tissues (e.g., DNA adducts, unscheduled DNA synthesis, single strand breaks).

Mutagenic effects other than those associated with direct DNA reactivity need to be carefully evaluated in regard to their mechanistic implications; some may have different cancer dose–response considerations than do direct DNA reactive agents. These effects include the ability of the chemical under review to induce indirect effects on DNA, such as through influence on the cell division spindle or production of reactive oxygen. Agents also should be evaluated for the presence of structural alerts that are often predictive of chemical reactivity or potential carcinogenicity. All of these findings are then melded into an overall appraisal of an agent's ability to influence genetic processes relevant to carcinogenesis in the thyroid or other sites.

It is possible that information on carcinogenic modes of action other than mutagenicity or thyroid–pituitary derangement will become available. If so, this information also needs to be incorporated on a case-by-case basis into the evaluation of a chemical's ability to produce tumors of the thyroid (and of other sites).

Dose–Response Considerations

Evaluation of potential dose–response relationships for thyroid tumors depends on an evaluation of the chemical's expected mode of carcinogenic action. Major determinations include whether the thyroid tumors appear to be due, at least in part, to thyroid–pituitary imbalance and whether other modes of action (e.g., relevant mutagenicity) may be pertinent to their formation. Other case-specific factors may provide crucial information, such as the extent to which data gaps and uncertainties prevail. A rationale should accompany the selection of any dose–response method. Guidance is provided below to illustrate ways to evaluate data and make judgments about potential thyroid cancer dose–response relationships.

High to low dose extrapolation. If antithyroid influences are operative in the formation of thyroid tumors, attention should be directed to biologically based procedures that embody the mechanistic influences, if they are available. In their absence, default procedures should be employed that incorporate nonlinear or linear considerations. When thyroid–pituitary imbalance is not operative, other modes of action or default considerations should be used; generally low-dose linear extrapolations are appropriate. All extrapolation procedures should be consistent with the guidance given in EPA cancer risk assessment guidelines. Finally, when thyroid tumors seem to arise from both a chemical's antithyroid activity and its mutagenic potential, dose–response relationships may be projected in ways that express concerns for both possible modes of action.

When tumors other than thyroid follicular cell (and related pituitary) tumors are found along with thyroid tumors, mode of action and other considerations should help guide the selection of the appropriate dose–response extrapolation method(s). Separate dose–response extrapolations may apply for the different tumor types, depending on the specifics of the case.

Biologically based. Optimally, mechanistic considerations that underlie thyroid tumor formation should be incorporated into biologically based extrapolation models. They should include physiologically based pharmacokinetic considerations for the chemical and its interactions with and effects upon cells. The trouble is that generic biologically based

dose–response extrapolation models have yet to be developed and validated for the thyroid. Fortunately, work in this area is beginning, and a model has been developed to explain effects associated with 2,3,7,8-tetrachlorodibeno-*p*-dioxin (*86*). Until other mechanistic models become generally available and chemical-specific data have been produced, the EPA assessments will employ, as discussed below, one of three default procedures in its evaluation of thyroid cancer risks: nonlinear, linear, or both nonlinear and linear.

Nonlinear. For cases in which tumors arise from chemically induced disturbances in thyroid–pituitary functioning, tumors are considered to be secondary to the adverse effects on thyroid gland function that precede them. As exposures to such agents decrease, the likelihood of cancer decreases; risks may be seen as minimal at doses at which there is no effect on thyroid–pituitary homeostasis. Generally, homeostasis is considered to apply when serum T_4, T_3, and TSH levels and thyroid and pituitary morphology and growth are within their normal limits. Risk assessments on agents should contain case-specific and generic information that support the contention that nonlinear dose–response relationships apply.

Empirically there is some support for thyroid follicular cell tumors having a dose–response curve that is less than linear (curvilinear upward). Slope functions were calculated using the Weibull model to give some indication of the shape of the dose–response curve and the rodent tumor incidence database of the National Cancer Institute/National Toxicology Program. Incidence for tumors in general was more consistent with a quadratic rather than a linear dose–response curve shape (*87,88*). However, when slopes for thyroid follicular cell tumors were compared with those of thyroid C cell tumors and all other tumor sites, those from the thyroid follicular cells were even more curvilinear than the others (Portier, personal communication).

One argument for assuming low-dose linearity in dose–response assessments is the concept of additivity to background. If a given chemical acts in the same way or augments an endogenous or exogenous background factor that contributes to tumor development, the effect of the chemical will add to that of the background factor. The result is low-dose linearity up to at least a doubling of the background rate (*89*). The concept may not be applicable to certain processes subject to hormonal regulation, such as with the thyroid gland. Normally the level of circulating thyroid hormone is adjusted carefully by the negative feedback with the pituitary. Elevations and reductions in thyroid hormone are met with adjustments in the amount of TSH released from the pituitary so as to bring thyroid hormone values back into the normal range. These excursions are not part of an ongoing carcinogenic process; instead, they represent the body's means of maintaining thyroid hormone homeostasis. Small doses of a potentially antithyroid chemical may not result in any perturbation in hormone levels or stimulation of thyroid follicular cell growth simply because homeostatic mechanisms will drive thyroid hormone levels back into the normal range.

The EPA acknowledges that it may be difficult to establish a precise dose where there is negligible response for a specific toxicologic effect given the sensitivity of methods to evaluate various parameters and the variability in measurement of end points. In recognition of this, it is incumbent upon the scientific community to help in the transfer of various molecular techniques from the research laboratory to testing facilities (e.g., measurement of hormone receptor mRNA production and receptor content, occupancy, and turnover) that may be more sensitive indicators of thyroid–pituitary malfunction.

The way the EPA has dealt with nonlinear phenomena is to express concern for human exposure (risk) as a margin of exposure (MOE), the ratio between a dose point of departure for the critical effect and the relevant estimate of anticipated human exposure (incorporating dose, frequency, and time). Large MOEs are attended with less concern than are small ones. Traditionally, the point of departure is expressed as a no observed adverse effect level (NOAEL), and the critical effect is the relevant toxicological end point occurring at the lowest doses in a toxicological study. More recently, alternatives to the NOAEL have been proposed to serve as points of departure for MOE calculation. In April 1996, the EPA proposed a revision of its existing cancer risk assessment guidelines (*2*). A dose point of departure is determined by extrapolating effects in the observed part of the dose–response curve. It is used, depending on the expected mode(s) of action, as the starting point for either linear extrapolation to the origin or calculation of an MOE in the case of nonlinear extrapolation. Generally, tumor or nontumor end point (e.g., hyperplasia) incidence is extrapolated to the 10% effect level. The 95% confidence levels are placed on that dose. The lower 95% confidence limit on that dose may be used as the point of departure for risk estimation below the observed data. There is some discussion about using the point estimate at the 10% effect level in lieu of the lower bound estimate in the proposal. Other means of determining departure points are also proposed (*2*). Final cancer guidelines will clarify these points. Procedures should be employed for thyroid tumors that are consistent with EPA cancer risk assessment guidelines and practices that are applicable at the time.

Assessments should include adequate information to aid in interpreting the significance of MOEs, such as taking into consideration the variability in sensitivity among individuals within a species, the sensitivity of humans relative to experimental animals, and other strengths, weaknesses, and uncertainties that are part of the assessment. Decision makers must then judge the adequacy of the MOE for their risk management purposes.

Low-dose linear. For those assessment cases in which thyroid tumors do not seem to be due to thyroid–pituitary imbalance (e.g., mutagenic agents), existing case-specific mode-of-action information and default considerations should be used to develop dose–response relationships. In other cases, there may be an absence of mode-of-action information for an agent. Generally a low-dose linear default for the thyroid tumors may be contemplated in these two circumstances in accordance with current EPA procedures. Recent cancer risk assessment guideline proposals suggest that linear extrapolation would involve calculation of a dose point of departure with a straight line extrapolation from there to the origin (*2*).

Low-dose linear and nonlinear. Finally, careful review is warranted when both antithyroid and other determinants seem to apply to the observed thyroid tumors, such as when there are certain mutagenic influences (e.g., structural chromosome aberrations). Judgments with accompanying scientific reasoning should be presented on the most appropriate way(s) to evaluate thyroid risk: either linear or nonlinear or, when the two positions are about equally tenable, both. When both procedures are presented, assessors should state the relative merits of each procedure. In some cases, one of the two methods may be preferable and should be given more weight; the rationale for conclusions should be expressly presented. Projected risks using linear extrapolation often give rise to concerns at doses lower than those when nonlinear techniques are applied. Thus, these two techniques usually can be seen as putting lower and upper bounds on exposures of concern. Assessments should include guidance for decision makers in interpreting concerns for exposure when both extrapolation techniques are presented.

End points to be employed. Optimally, one would have access to data on various preneoplastic end points that would be evaluated following short-term (e.g., 2–4 week)

and subchronic (e.g., 13 week) studies and compared with the doses that have produced tumors in chronic studies. End points that should regularly be evaluated and presented in dose–response analyses include 1) changes in levels of T_4 and T_3; 2) increases in TSH; 3) the incidence of thyroid follicular cell hypertrophy, hyperplasia, and neoplasia; 4) increases in cell proliferation and thyroid weight; and 5) specific end points associated with thyroid–pituitary disturbance at the site(s) of chemical action (e.g., inhibition of thyroid peroxidase, increased metabolism and clearance of thyroid hormone). A host of other effects as discussed above could be monitored in the thyroid, pituitary, or thyroid hormone-responsive organs and included on a case-by-case basis. Care should be taken to ensure that studies to evaluate these parameters are conducted for adequate periods of time and at doses that clearly define dose–response relationships. Attention also needs to be given to procedures that help to reduce the variability in responses among animals (e.g., time and means of animal sacrifice and tissue sampling).

Estimation of points of departure. For the important toxicity studies, a point of departure (e.g., NOAEL) is determined for each thyroid toxicity end point and exposure duration. Doses associated with tumors should also be noted. The departure point may be a study dose or an estimated dose. For instance, when data permit, the departure point can be estimated by extrapolation of doses associated with observed responses (e.g., TSH levels) to those attended with no significant deviation from the control range. In other cases, an appropriate observed study NOAEL may be selected or other procedures in accordance with EPA guidance may be used.

Considerations for the selection of the critical end point to be used to project thyroid cancer risk (i.e., calculation of the MOE) include 1) the nature of the end point and its relationship to the perturbations in endocrine balance and carcinogenicity; 2) the presence of good dose–response or dose-severity of effect relationships; 3) the sensitivity of the end point vis-à-vis other potential end points; and 4) the length of the dosing period and its relevance to making judgments about the consequences of potential chronic exposures.

Interspecies Extrapolation

Many considerations are relevant in attempting to extrapolate thyroid carcinogenic effects in experimental animals to humans. The relative sensitivity of humans and rodents to the carcinogenic effects of elevated TSH are not firmly established, but important observations have been made. Given that the rodent is a sensitive model for measuring the carcinogenic influences of TSH and that humans appear to be less responsive, one would expect that projections of potential risk for rodents would serve as conservative potential indicators of risks for humans.

Rodent cancer studies typically include doses that lead to toxicity, including perturbation in thyroid–pituitary functioning, over a lifetime. The relevance of such experimental conditions to anticipated human exposure scenarios (i.e., dose, frequency, and time) should be considered and presented in the final characterization of risk. This is especially true because thyroidal effects are not necessarily expected at all doses. In addition, chemically induced effects that are produced by short-term disruption in thyroid–pituitary functioning appear to be reversible when the stimulus is removed.

Although it appears that humans are less sensitive to the carcinogenic perturbations of thyroid–pituitary status than rodents (e.g., iodide deficiency), such determinations should be made on a case-by-case basis. This would depend upon a host of factors involving the agent, including the depth and breadth of the database, the congruence of the information supporting a given mode of action, and the existence of information on humans. Decision makers should be apprised of risk assessment judgments and their rationales. In the absence of chemical-specific information, the default position is that humans should be considered to be as sensitive to carcinogenic effects as are rodents. That is to say, a factor of one would be used when extrapolating effects in rodents to those in humans.

Human intraspecies evaluations. Thyroid hormones are regulated within rather narrow ranges. Normal human serum values are as follows: T_4, 4–11 μg/dl and T_3, 80–180 ng/dl; TSH levels extend over a broader range: 0.4 to 8 μU/ml due to the incorporation in recent years of more sensitive laboratory methods that have extended the normal range to lower values (*90,91*). To the extent that data exist in humans as to their thyroid–pituitary status, one should carefully review central value parameters between chemically exposed and unexposed groups as well as the distribution of values between both groups.

Extended deviations in human thyroid hormone levels either above or below the normal range are associated with the disease states hyperthyroidism and hypothyroidism, respectively. In the United States, most cases are associated with some autoimmune problem. Worldwide, iodide deficiency is the most prominent cause of thyroid disease generally and hypothyroidism specifically. People develop characteristic symptoms and signs that readily cause them to seek medical attention. The goal of therapy for such conditions is to bring persons back into normal thyroid–pituitary balance, which secondarily would greatly minimize any potential for carcinogenic effects. Overt hypothyroidism, with reduced thyroid hormone and increased TSH levels, requires treatment; it has an incidence of about 0.2% in women and less in men. Subclinical hypothyroidism may have an incidence of 5% among women. Men are affected less often; incidence increases significantly with age. There is not agreement as to whether these people need treatment (*92*). Some with hypothyroidism may go for some time before diagnosis and treatment. The possible consequences of chemical exposure on this subpopulation of individuals may warrant consideration.

The human thyroid is susceptible to ionizing radiation, the only known human thyroid carcinogen. Children are more sensitive than adults to the carcinogenic effects of ionizing radiation (*41,53*). In keeping with the EPA position on the evaluation of childhood risks (*93*), the impact of mutagenic chemicals that produce thyroid tumors should be evaluated in context of the information available on the subject as well as agent-specific information. The extent to which a chemical may act as ionizing radiation does should be evaluated on a case-by-case basis and factored into decisions accordingly. It seems possible that chemicals producing mutagenic effects like those of radiation may pose some accentuated risk to children. More research is needed in this area (*94*).

Combined effects. It is not known if there are human subpopulations that are sensitive to thyroid cancer development from exposure to antithyroid chemicals. Seemingly, the thyroid status of persons living in an iodide-deficient area or those who are hypothyroid due to other causes may further be harmed by simultaneous exposure to naturally occurring chemicals or xenobiotics that can further disrupt thyroid–pituitary functioning (*95*). This potential to possibly increase cancer risk following chemical exposure should be kept in mind in evaluating the composition of human populations, the numbers and nature of sensitive individuals, the magnitude and pattern of chemical exposure, estimates of risk to the general population, and the potential risk consequences to persons with some increase in sensitivity.

In addition to possible sensitive subpopulations, persons may be exposed to more than one antithyroid chemical at a time and by more than one route of exposure. These combined exposures may worsen thyroid–pituitary status and influence thyroid cancer potential (*96*). To the

extent practicable on a case-by-case basis, consideration should be given to combined exposures to chemicals that influence thyroid–pituitary functioning. Attention can be directed toward 1) an understanding of whether the chemicals affect the same or different sites of antithyroid action, 2) whether mutagenicity or other modes of action may be operative, and 3) the ways various antithyroidal and other effects may combine to influence potential cancer risks by the same and different routes of exposure. Some guidance for evaluating these cases is provided in the EPA mixtures assessment guidelines (*97*).

Unlike tumors at many sites, modes of action for thyroid follicular cell tumors can be readily explored through laboratory experimentation. By using this cancer mode-of-action information along with considerations about intraspecies and interespecies variability, chemicals can be assessed as to the consequences of human exposure.

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